

Effects of wind on movement of *Diachasmimorpha longicaudata*, a parasitoid of tephritid fruit flies, in a laboratory flight tunnel

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Abstract

Cohorts of mass-reared adult female *Diachasmimorpha longicaudata* (Ashmead), parasitoids of tephritid fruit fly larvae, were released in a laminar air flow wind tunnel to study the effects of air movement on flight and walking behaviors. Wind in the tunnel was cycled on and off at intervals to simulate gusty conditions observed in the field. Wind speed influenced parasitoid movement during both the wind-on periods and during the calm intervals between gusts. Wind speeds of 0.8 m per second suppressed flight and walking behaviors, while wind speeds of 0.4 m/s stimulated both flight and walking behaviors. There was an exponential increase in the ratio of activity in calm to activity in wind as wind speed increased.

Introduction

In recent years a number of entomologists have attempted to use augmentative releases of mass-reared opiine braconid parasitoids to control field populations of tephritid flies in tropical fruit and vegetable crops (Wong et al., 1991, 1992; Sivinski et al., 1996). While numerous studies have been conducted to obtain bionomic data and establish rearing methods for these parasitoids (Ramadan et al., 1989a,b; Wong & Ramadan, 1992; Messing et al., 1993), relatively little work has been done on the behavioral parameters which are critical to augmentation success. For example, for the most widely tested opiine species, *Diachasmimorpha longicaudata* (Ashmead) (Hymenoptera: Braconidae), a larval parasitoid of both Old and New World fruit flies, we know very little about flight behavior, dispersal capabilities, and response to abiotic factors. While a few augmentation projects using opiine braconids have reported successful reductions of fruit fly infestations (Wong et al., 1991; Sivinski et al., 1996), other projects have failed, for reasons that remain largely unknown. Thus, it is difficult to determine which factors lead to success or failure, and to go beyond empiricism to a more thorough under-

standing of the conditions favorable for augmentation success.

During the course of experiments designed to investigate stimuli used in parasitoid host-habitat finding (Messing & Jang, 1992), we observed that *D. longicaudata* flight behavior appeared sensitive to fluctuations in wind intensity, both in the laboratory and the field. We therefore conducted a series of wind tunnel experiments to examine this response in more detail. Our objectives were to determine the effects of alternating periods of wind and calm (reflecting gusty conditions observed in the field), and of wind speed on flight behavior of mass-reared adult females. Since adult parasitoids search for hosts (fruit fly larvae) on plant substrates by walking, and were frequently observed to move by walking in the wind tunnel, we also analyzed the effects of the variables on walking behavior.

Materials and methods

Parasitoid rearing. Parasitoids were reared at the USDA-ARS Tropical Fruit and Vegetable Research Laboratory in Honolulu according to the methods of Wong & Ramadan (1992). Cohorts of parasitized oriental fruit fly, *Bactrocera dorsalis* (Hendel) puparia

were shipped via air freight weekly from Oahu to Hilo, Hawaii. Puparia were then placed in $30 \times 30 \times 30$ cm wooden screened cages and allowed to emerge. Male and female parasitoids were held together in these cages at 22–25 °C, 55–70% r.h., and indirect natural lighting from a 1.5 m² glass window reflected off white painted walls. Honey and water were provided *ad libitum*.

Cage trial. In initial tests we examined the behavior of *D. longicaudata* females in $30 \times 30 \times 30$ cm wood and screen cages. A 30 cm diam. electric fan was placed on a separate table level with the cage at a distance of 2.0 m. When the fan was turned on it generated wind inside the cage at a speed of 0.3 m/s. For each trial, ten 7–14 d old parasitoid females were aspirated from the maintenance colony into a 20 ml plastic vial. The vial was placed on the floor of the test cage for five minutes to allow the parasitoids to acclimate, then gently opened by hand to begin the test. Each test lasted 27 min. The fan was left off for the first 6 min, then turned on and off at 3 min intervals until 21 min, then turned off again for the final 6 min (see Figure 1). An observer recorded the total number of flights for all wasps in the cage during each minute of the 27 min trial. All tests were done in the laboratory between 09:00–14:00 h, at ambient temperature and humidity, with natural light coming through six 1.5×1 m windows. The test was replicated 10 times.

Wind tunnel trials. A series of more finely controlled tests was then conducted in a laminar airflow wind tunnel, as described in Jang & Light (1991). The tunnel was constructed of tempered-glass and measured 85.5 wide \times 86.5 cm tall \times 261 cm long. Intake and exhaust fans were housed at both ends of the glass in galvanized sheet metal ducts. To achieve maximum air flow (ca. 0.9 m/sec) through the wind tunnel, the frame was separated from the exhaust duct and only the intake fan was used in our experiments. An aluminum-framed screen was placed transversely across the long axis of the tunnel at the upwind end to exclude parasitoids from the fan housing.

The rate of laminar airflow through the wind tunnel during each trial was adjusted using a 0–140 volt rheostat (model 3PN1010, Staco Energy Products, Dayton, OH) connected to the intake fan. Measurements of wind speed were taken with a portable air velocity meter (model 1650, TSI Inc. St. Paul, MN) at the center of the intake hood, 10 cm above the floor. The wind speed was measured at the beginning and end

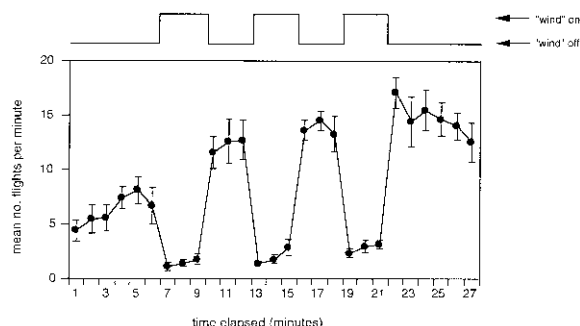


Figure 1. Flight response of 10 caged female *D. longicaudata* to 0.3 m/s wind pulses in a laboratory cage.

of each trial and was found to vary only slightly (± 0.02 m/s within 1 min). The absolute mean change in wind speed over the 40 min duration of each test was $|0.04| \pm 0.01$ m/s.

Two 1.2 m long fluorescent light bulbs were placed 18 cm above the upwind end of the wind tunnel, providing 450 lumens of light during each trial (as measured on the floor of the tunnel directly beneath the bulbs). Although the laboratory was air conditioned and dehumidified, temperature and relative humidity in the wind tunnel still varied because of extreme weather conditions outdoors (e.g., outside r.h. varied between 50–99%). Temperature and humidity in the room outside the tunnel were therefore recorded at the beginning and end of each test.

For each trial in the wind tunnel, 6 female parasitoids were aspirated into a 20 ml plastic vial and carried from the rearing room to the laboratory. Vials were plugged at one end with a plastic stopper, and had 1.5 m of plastic tubing extending from a hole at the other end. To begin each trial, the vial was placed on the center of the floor of the wind tunnel and parasitoids were allowed to acclimate to wind tunnel conditions within the vial for 10 min before being released by blowing gently on the tubing to eject the stopper. Although this is a relatively short acclimatization period, conditions in the wind tunnel were fairly similar to conditions in the parasitoid holding room, and the duration of each trial (40 min) was sufficient for parasitoids to acclimate and respond to variations in wind speed.

Each trial lasted for 40 min. An electronic timer (ChronTrol XT, ChronTrol Corp., San Diego, CA) was used to turn the intake fan on and off at intervals of 60 s. There were thus 20 one-minute 'wind' periods and 20 one-minute 'calm' periods during each trial. It required ca. 6 s after switching the power on for the fan

to attain maximum speed, and there was a 2 s lag time between cutoff of power to the fan and actual cessation of movement of the fan blades. For the purposes of analysis, nominal on-off times were used.

At the beginning of a calm period, parasitoids were expelled into the wind tunnel by gently blowing on the plastic tubing. Measurements of activity began at the start of the next wind period. We measured two distinct behaviors during each trial, flights and walks. Flights were defined as any movements, however large or small, in which a parasitoid lifted all six legs from the glass substrate and became airborne before landing again. *D. longicaudata* rarely hops or jumps, so this definition allowed us to enumerate all flight behaviors regardless of orientation or duration. A walk was defined as any single continuous movement during which a parasitoid moved in a forward direction at least one body length before coming to a complete stop. Flights and walks were recorded by an observer sitting 1 m from the side of the wind tunnel. All data were recorded on a computer using Observer[®] behavioral observation software (version 2.0, Noldus Information Technology bv, Wageningen, The Netherlands).

Four wind speeds were tested: 0.1, 0.2, 0.4, and 0.8 m/s. These speeds were randomized among four daytime intervals: 8:00, 10:00, 12:00, and 14:00 h. Experiments were conducted with 7, 8, 9, 11, and 14 day old females. Trials were repeated until at least two replications for every wind-speed/ time of day combination were completed, but not all permutations of the variables (parasitoid age, time of day, relative humidity, and temperature) were replicated equally.

Correlation analysis (PROC CORR: SAS, 1988) and plots of parasitoid response (both flights and walks) indicated that wind speed had a non-linear relationship to activity. Therefore, second order polynomials were used in SAS NLIN to find best fit models for mean activity/ wasp/ min. as a function of wind speed (separately for flights and walks, with and without wind). The correlation of predicted values of the model to actual data was performed using PROC CORR (SAS, 1988). Spearman's rank correlations (R_s) are presented. The NLIN procedure was also used to derive an exponential model of the ratio of activity in wind to activity in calm as a function of wind speed.

Results and discussion

Cage trial. Although wind speed in the initial trials in screened cages was only 0.3 m/s, the parasitoids showed a clear response to this wind in the number of

flights taken within the confines of the cage (Figure 1). Wasp cohorts averaged 5–8 flights/min during the initial calm period, but the number of flights decreased abruptly to < 2 flights/min during the first wind period. Subsequent cycling of the wind off and on resulted in a very regular response by the parasitoids, with > 10 flights/ min occurring during calm periods, and < 3 flights/min during wind periods.

Wind tunnel trials. Wind speed in the flight tunnel had a significant influence on the number of parasitoid flights both during the intervals when wind was blowing, and during the intermittent calm periods (Figure 2). Increasing wind speeds appeared to stimulate flight activity up to about 0.4 m/s, but when the speed approached 0.8 m/s flight activity was suppressed. This occurred even though parasitoids were occasionally observed in preliminary pilot tests to be physically capable of remaining airborne and flying in 0.8 m/s wind. Interestingly, walking behavior was also affected by wind speed (Figure 2). The pattern of response for walking was similar to that of flight activity, except that walks were approximately ten times more frequent than flights.

For both flights and walks, there was an exponential increase in the ratio of activity during calm intervals to that during wind intervals as the wind speed increased (Figure 3). As wind speed approached 1 m/s there was about 5 times more activity in calm than in windy periods. If this model is extrapolated to higher wind speeds, then flight and walking behavior would virtually cease during wind gusts of only a few meters per second.

The parasitoids used in this study were mated, well-fed, host-deprived females that would be expected to respond to any stimulus which could be used to orient towards habitats with hosts suitable for oviposition. It has been shown in several previous studies that *D. longicaudata* responds by positive anemotaxis to wind-borne odors of several host fruits (Greany et al., 1976; Messing & Jang, 1992). However, there were no fruit odors present in the wind tunnel (nor in the wind tunnel room) and it seems clear that the effect of wind on parasitoid movement had no olfactory basis. Rather, the direct physical stimulation of moving air appears sufficient to induce the parasitoids' flight, which may represent initial dispersal or host-habitat finding movement.

The success of augmentative biological control programs is dependent on the ability of mass-reared parasitoids to disperse from release vessels under natural conditions and to locate their hosts in the field. Adult

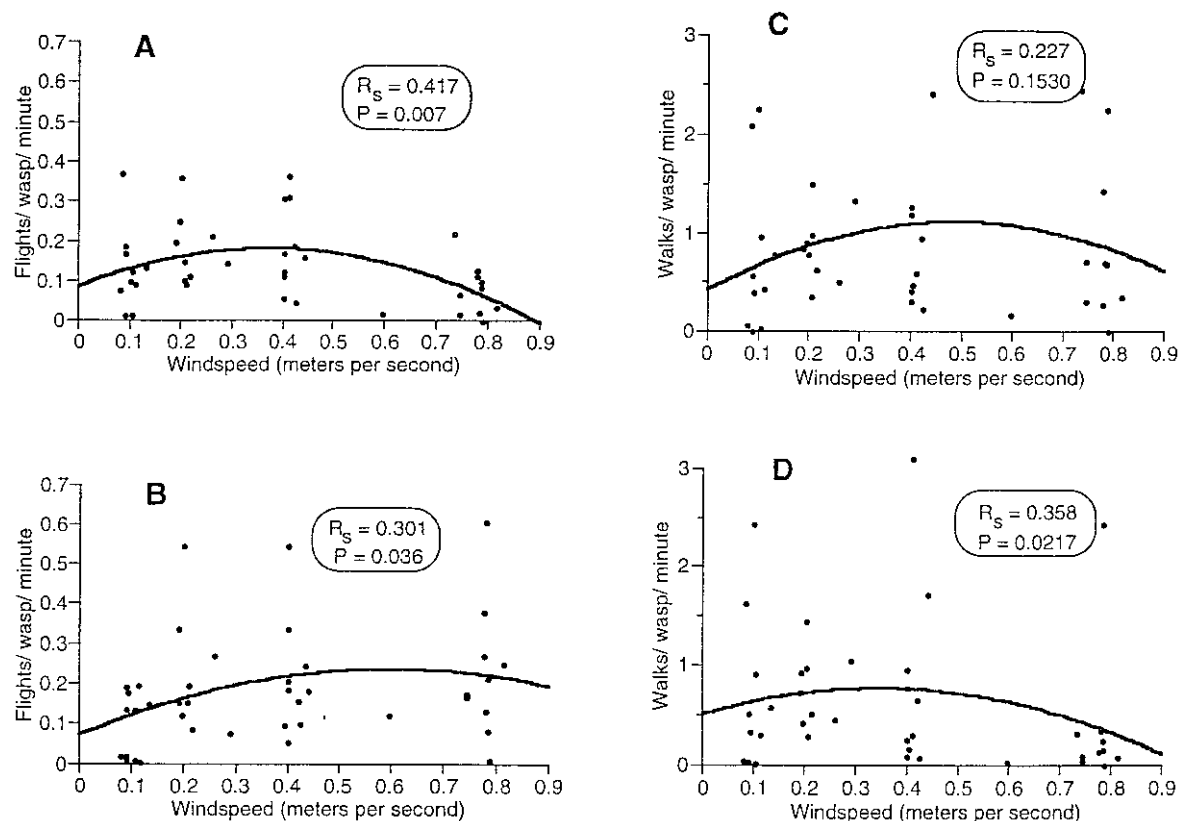


Figure 2. Effect of wind speed on movement of *D. longicaudata* in a laminar airflow wind tunnel: (A) flights in wind; (B) flights in calm; (C) walks in wind; (D) walks in calm.

parasitoid behavior from the time of release until oviposition is influenced by a number of interacting biotic and abiotic factors. Much of the discussion in the literature concerning this behavior has focused primarily on biotic factors, such as visual and olfactory stimuli important in orientation (Messing & Jang, 1992), the effects of experience and learning on host location and host preference (Turlings et al., 1993), and state variables such as egg load (Rosenheim, 1993). Relatively few workers have addressed the effects of abiotic environmental factors on parasitoid movement and host finding.

Our data show that the flight behavior of *D. longicaudata*, at least under the confined laboratory conditions described above, is very sensitive to even modest wind speeds. This corroborates observations of workers in the field, who noticed that when mass-reared *D. longicaudata* were released under natural conditions, the wasps tended to remain on the rim of the release vessel during even the slightest breeze, and to take off (in a downwind direction) during the intervals

between wind gusts. The indoor cage trial (Figure 1) demonstrates that the parasitoids readily perceive, and respond to, air movements of 0.3 m/s, and show a pronounced tendency to abstain from flight even at this low wind speed. The wind tunnel trials confirm this response of wasps to low wind speeds, and further indicate that the air movement, which inhibits flight while the wind is blowing, actually stimulates flight during intermittent calm periods (i.e., there are more flights per minute in the calm intervals between 0.4 m/s wind gusts than in the calm intervals between 0.1 m/s gusts; Figure 2B).

In all cases of parasitoid augmentation with opiine braconids used against tephritid fruit flies, the parasitoids employed are exotic species which were originally imported into a region during classical biological control programs. Establishment of the parasitoid population in the new region indicates a generally successful adaptation to the new climate and a proven ability to find enough hosts to reproduce and persist in the new environment. However, the more subtle effects of

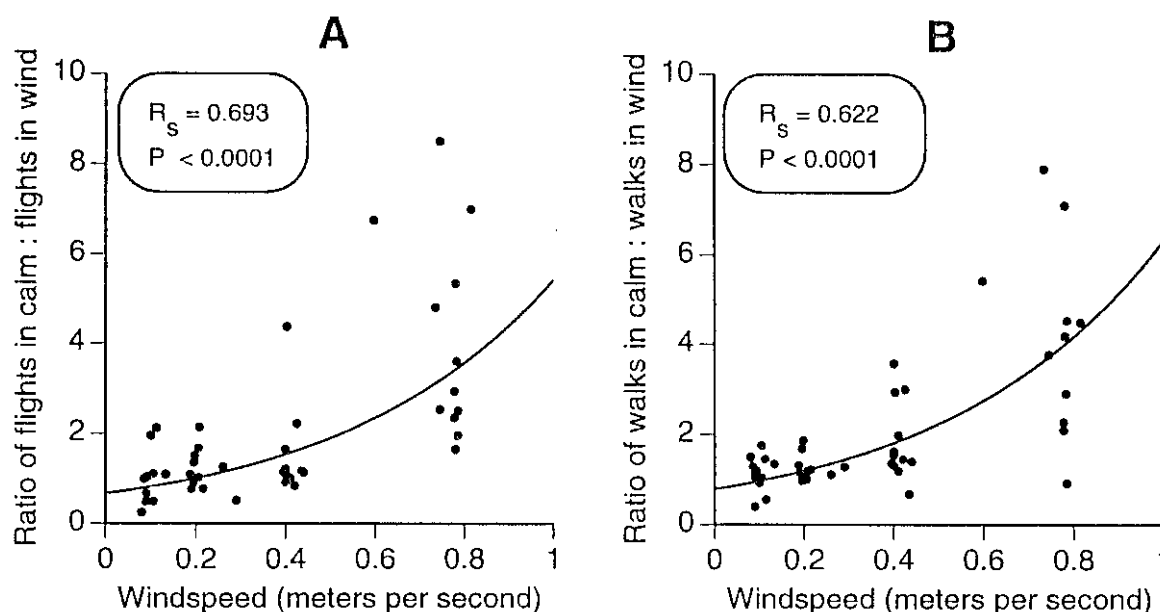


Figure 3. Effect of wind speed on the ratio of *D. longicaudata* activity in the wind to activity in the calm in a laminar airflow wind tunnel (A) = flights; (B) = walks.

the new climate on parasitoid behavior and efficacy in reducing host populations have rarely been examined. The wind tunnel studies reported here provide a first step in examining the effect of environmental factors on post-release movements of the most widely used fruit fly parasitoid worldwide.

Many species of parasitoids locate hosts and host habitats via anemotactic responses to airborne odors emanating from host insects or their host plants (Tumlinson et al., 1993; Vinson, 1976). This behavior has been well demonstrated in several opiine parasitoids which attack tephritid fruit flies (Greany et al., 1977; Levy et al., 1991; Messing & Jang, 1992; Messing et al., 1996). Parasitoid response to wind in the field is thus complex, representing reaction to both a direct physical stimulus (as shown in our wind tunnel experiments) as well as olfactory stimuli carried in air currents. The response is further complicated by variables such as parasitoid age, egg load, and prior experience. For *D. longicaudata*, absence of wind would make host finding difficult due to a resultant lack of directional olfactory cues over any appreciable distance, while strong winds have an inhibitory effect on flight (Figures 2a, b). Thus it appears that gentle, intermittent winds are most favorable for dispersal and host habitat finding in this species. Stimuli used for finding host larvae (i.e., vibrations from within fruit:

Lawrence, 1981) are not wind borne, so lack of air movement would not interfere with host location, but strong wind does inhibit walking (Figures 2c,d), and thus may interfere with host finding behavior.

D. longicaudata has been used in separate augmentative release projects by workers in Costa Rica (Messing, 1996); Florida (Sivinski et al., 1996); Hawaii (M.F. Purcell, pers. comm.); Mexico (R.A. Wharton, pers. comm.); Spain (Jimenez & Castillo, 1992); and Taiwan (Yao, 1989). In most of these cases, *D. longicaudata* was chosen because it is the easiest opiine parasitoid to rear, giving the highest yields per unit of parasitized host puparia in the insectary. However, no convincing data have been presented to show that this species is more effective in the field than other imported species, or than additional parasitoids yet to be imported from their areas of endemism. Data from these wind tunnel experiments suggest that *D. longicaudata* has a somewhat reduced flight and walking efficiency under conditions which are common in some agricultural areas of Hawaii (i.e., northeast trade winds attain speeds of over 5.8 m/s more than 50% of the time on Kauai, Oahu, and Maui: Armstrong, 1983).

During the 1950's, Hawaii had a wide-ranging classical biological control program directed against tephritid fruit flies, resulting in the establishment of at least six species of opiine parasitoids in the islands

(Wharton, 1989). However, no additional species have been introduced in the forty years since that time. It is well known that many additional parasitoid species exist that attack tephritid fruit flies in tropical and sub-tropical parts of Africa, Asia and South America (Wharton, 1989), and it is quite possible that some of these species may be better adapted to Hawaiian conditions than the ones which are currently established. Behavioral tests such as the wind tunnel study reported here may provide valuable information as to which of these species would be most promising for future introductions and releases in particular habitats.

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